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Analysis of IgG with specificity for variant surface antigens expressed by placental *Plasmodium falciparum* isolates

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Abstract

Background: Pregnancy-associated malaria (PAM) is caused by *Plasmodium falciparum*-infected erythrocytes that can sequester in placental intervillous space by expressing particular variant surface antigens (VSA) that can mediate adhesion to chondroitin sulfate A (CSA) *in vitro*. IgG antibodies with specificity for the VSA expressed by these parasites (VSA_{PAM}) are associated with protection from maternal anaemia, prematurity and low birth weight, which is the greatest risk factor for death in the first month of life.

Methods: In this study, the development of anti-VSA_{PAM} antibodies in a group of 151 women who presented to the maternity ward of Albert Schweitzer Hospital in Lambaréné, Gabon for delivery was analysed using flow cytometry assays. Plasma samples from placenta infected primiparous women were also investigated for their capacity to inhibit parasite binding to CSA *in vitro*.

Results: In the study cohort, primiparous as well as secundiparous women had the greatest risk of infection at delivery as well as during pregnancy. Primiparous women with infected placentas at delivery showed higher levels of VSA_{PAM}-specific IgG compared to women who had no malaria infections at delivery. Placental isolates of Gabonese and Senegalese origin tested on plasma samples from Gabon showed parity dependency and gender specificity patterns. There was a significant correlation of plasma reactivity as measured by flow cytometry between different placental isolates. In the plasma of infected primiparous women, VSA_{PAM}-specific IgG measured by flow cytometry could be correlated with anti-adhesion antibodies measured by the inhibition of CSA binding.

Conclusion: Recognition of placental parasites shows a parity- and sex- dependent pattern, like that previously observed in laboratory strains selected to bind to CSA. Placental infections at delivery in primiparous women appear to be sufficient to induce functional antibodies which can both recognize the surface of the infected erythrocytes as well as block their binding to CSA. The correlation between serum reactivities of placental field isolates from different geographic locations and collected at different times is indicative of the conserved nature of the antigen(s) mediating PAM.

Background

Adults living in malaria endemic areas have normally been exposed to repeated infections with *Plasmodium falciparum* and are, therefore, clinically immune to the disease [1,2]. However, this is not true in the case of pregnant women and the disease is more pronounced in primigravid women than in multigravidas. Pregnancy associated malaria (PAM) may be accompanied by maternal anaemia, abortion, stillbirth, prematurity, intrauterine growth retardation and low birth weight, this being the greatest risk factor for death in the first month of life [3].

It is now known that malaria-infected individuals living in endemic areas accumulate a broad spectrum of protective antibodies directed against variant surface antigens (VSAs) present on infected erythrocytes (IEs) [4,5]. Further studies show that agglutinating antibodies are principally directed against the major surface antigen *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) [4,6]. Severity of malaria is related to the capacity of the *P. falciparum* IEs to cytoadhere and to sequester in the microvascular capillaries of vital organs [7].

In pregnant women, the placenta provides a new opportunity for the selective accumulation of *P. falciparum* IEs [8]. IEs appear to express a different PfEMP-1 molecule capable of interacting with chondroitin sulfate A (CSA), a glycosaminoglycan (GAG) in the placental intervillous space [8]. Other potential receptor molecules in the placenta, such as hyaluronic acid and non-immune immunoglobulin G, are also known to interact with IEs [9,10]. Anti-VSA antibodies accumulate with each pregnancy and protect the mother from placental malaria in subsequent pregnancies [11,12]. The targets of these antibodies are thought to be generated in a strain-independent manner, with the capacity of recognizing parasites from different geographic locations. The panreactive anti-CSA binding antibodies also react with the surface of IEs in a sex-specific manner, such that serum samples from malaria-exposed males do not show any reactivities toward IE surface [13,14]. In one study, in which placental isolates were used, it was shown that primigravid women lacked antibodies that were capable of inhibiting the adhesion of IEs to CSA [13], whereas in a second study where they used a parasite selected on CSA, a significant level of anti-adhesion antibodies was detected in primiparous women whose placentas were found to carry malaria infections at delivery [15]. Agglutinating antibodies were also detected in primiparous women presented with infected placentas but were not correlated with their capacity to inhibit CSA adhesion [16]. In another study, significant association between CSA-adhesion inhibitory antibodies and agglutination antibodies was reported, but many samples were found to differ in their quality, on the one hand in strongly inhibiting adhesion, but on the other in being negative or only weakly positive in agglutination [17].

In addition to agglutination and anti-adhesion assays, flow cytometric analysis has also been used successfully to detect antibodies in sera from pregnant women directed against the surface of *P. falciparum* IEs, previously selected to bind CSA [14]. Here we describe the detection of VSA_{PAM}-specific IgG in the plasma of a group of pregnant women by flow cytometry using a panel of placental parasites. Antibody reactivities were assessed relative to parity and gender, comparing infection rates of women with different parities. Antibody levels with respect to malaria infections during the course of a pregnancy were also analysed. To gain an insight into the nature of these antibodies, we also sought to determine whether VSA_{PAM}-specific IgG measured in flow cytometry can block parasite binding to CSA.

Materials and Methods

Study participants

One hundred and fifty-one women presented at the maternity ward of the Albert Schweitzer Hospital in Lambaréné, Gabon for delivery between May and October 2002. Whenever possible, demographic data (e.g. ethnic group, date of birth, weight) were collected from the study participants upon admission. For those attending prenatal care, information regarding malaria infections during the course of pregnancy were taken from the maternity record book, which documented peripheral parasitemia by thick blood smears. Written informed consents were obtained from all of the women and the study was approved by the ethics committee of the International Foundation of the hospital.

Blood samples

Peripheral blood was collected from the study participants within one hour after delivery in an EDTA-containing monovette and used in thick blood smears to determine parasite densities. Giemsa-stained slides were examined by light microscopy covering 100 fields with the l00× objective under oil immersion and parasite densities were recorded [18]. Peripheral blood samples were then centrifuged (2,000 × g for 10 min) to obtain the plasma and used in flow cytometric assays.

Blood was also extracted directly from the placenta. This was done by excising a block of placental tissue (2 cm^3) from the maternal side of the placenta, placing it into a 50 ml falcon tube containing 25 ml of RPMI 1640 medium and leaving it to drain on a horizontal rotator for about 10 min. Thereafter, the tissue block was squeezed with a pair of forceps and removed from the tube. The remaining solution was centrifuged (2000 × g for 10 min) and the

pellet was examined for the presence of malaria parasites by conventional microscopy, as described above.

Placental parasite isolation

Placental blood for subsequent parasite culture was extracted from thick blood smear positive placentas. First, several one-centimeter deep incisions in the mother side of the placenta were made and blood was withdrawn into an EDTA syringe (monovette without needle). Gentle squeezing of the placenta was found to help increase the blood volume. Typically, three to six mls of blood were obtained. Leucocytes were removed by Ficoll treatment and the remaining cells were washed twice in RPMI 1640 medium. The cell pellet was resuspended to 5 % haematocrit in RPMI 1640 medium supplemented with 0.5 % Albumax II, 2 % human serum and 200 μM hypoxanthine and placed in a tissue culture flask at 37°C in a candle jar. Parasitized erythrocytes were left to grow for one or two cycles until the early ring stage before freezing. Parasites were frozen in several aliquots using the Glycerolyte method and thawed using the 1.6 % and 12 % NaCl method [19]. Parasite CSA adhesion phenotype usually diminished after about four weeks of continuous culture. Consequently, enrichment of the phenotype on CSA was performed every three weeks to maintain high levels of binding to CSA [20].

Placental isolates used in this work were taken from two different study cohorts, two parasites (Gb337 and Gb218) from Lambaréné, as previously described [21] and two parasites (vip43 and vip42) from Senegal. In Senegal, the study took place in Pikine-Guédiawaye, located 15 km north-east of Dakar, where malaria is hypoendemic with an average of one infectious bite/person/year. Malaria transmission is highly seasonal during the rains, from September to January [22]. The study took place in the maternity wards of the Centre de Santé Roi Baudoin de Guédiawaye with nearly 7,000 childbirths per year. Pregnant women were enrolled after informed consent was obtained in the delivery room from October 2001 to March 2002. This study was approved by the National Ethics Committee of the Senegalese Health Ministry. To allow the identification of women with malarial infection, an immunochromatographic test (ICT) [Sarachim, Lausanne, Switzerland] was performed on finger pricked capillary blood before delivery. Those presenting with an infection were enrolled. The placenta was collected at delivery for parasite isolation and a placental thick blood smear was made for microscope examination [23].

Flow cytometry assays

The plasma samples from our study cohort were examined by flow cytometry for recognition of specific antigens localized on the surface of IEs, as described previously [24]. Plasma samples from five nulligravid women and from seven males living in the same endemic area served as controls. Late-stage IEs were enriched by magnet-activated cell sorting (MACS), diluted to approximately 2×10^6 IEs/ml in PBS-2% FCS, and stained with 2 μg/ml of ethidium bromide. The suspension (100 μl) was mixed with 5 μl of human plasma and incubated at 4°C for 30 min. Cells were washed twice each with 3 ml of PBS-2% FCS. Subsequently, cells were stained with two layers of antibodies, first with a goat anti-human IgG (diluted at 1:250), followed by a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-goat Ig (diluted at 1:25), with a washing step between each antibody incubation, as described above. Bound human IgG was quantified and expressed as the mean FITC fluorescence intensity (MFI) of ethidium bromide-gated IEs using a FACSCAN flow cytometer.

Inhibition of binding assays

CSA binding plates (Falcon; catalogue no. 351016) were prepared by drawing up to 10 circles of about 2 mm radius on the plates using a Dakocytomation pen. Two of the 10 spots on each plate served as control, where no plasma was added. In this way, up to four different plasma samples in duplicate can be tested on one plate at any one time. The procedure was carried out by adding 7 μl of a CSA solution (100 μg/ml dissolved in PBS) to the middle of each circle and the plates were kept moist by leaving the lid on and left at room temperature for one hour. The CSA solution was aspirated from each circle and free protein-binding sites were blocked for 30 min using a PBS-1% BSA solution. Plates prepared in this way were used immediately. Binding inhibition assays were performed as follows: late-stage IEs were enriched by MACS, 5 μl of human plasma were added to 100 μl of approximately 10^7 IEs/ml diluted in PBS-2% BSA, and incubated for one hour at 37°C. Ten μl of the cell suspension were placed on the CSA spot and incubation continued for another hour at 37°C in a humid incubator. The plates were washed twice in the presence of PBS and the bound cells were fixed with 2 % glutaraldehyde for 30 min and stained with Giemsa. The number of bound cells per mm^2 was counted under the light microscope at low power and the average of 4 counts was taken as the number of bound cells/mm^2.

Statistical analysis and data presentation

Groupwise medians were compared by Wilcoxon rank test (for two groups) and Kruskal-Wallis one-way analysis of variance (for more than two groups). Spearman's test (r_s) was used for analysis of parameter association.

Results

Comparison of placental parasitemia by thick blood smear

From the group of 151 women enrolled in the study, 41 were categorized as primiparous women, 23 were secundiparous women and 87 were multiparous women. Thick

Table 1: Placental thick blood smear results and documented peripheral parasitaemia during pregnancy data of women included in the study

Parity	Placenta positive at delivery	Peripheral blood positive during pregnancy	Double postive
Primiparous (n = 41)	8 (20 %)	9	2
Secundiparous (n = 23)	4 (17 %)	4	0
Multiparous (n = 87)	6 (7 %)	5	0

blood smear analysis of their placental blood showed that 11% of the study participants were infected at delivery, with the primiparous and secundiparous women showing higher infection rates (20% and 17%, respectively), compared to multiparous women (7%) (Table 1). A comparison was also made between the presence of infections at the time of delivery and during the course of the pregnancy. In the primiparous group, nine women had peripheral parasitaemia during their pregnancies, whereas two women were infected both at delivery and during their pregnancies. The risk for a woman pregnant for the first time to be infected during her pregnancy and at delivery was higher than the similar risk for a woman in her further pregnancies. This conclusion is supported by the observation that in the secundi- and multiparous groups, no woman who was infected at any time during the entire period of pregnancy was also infected at delivery.

To further analyse placental infection rates in our study participants at the time of delivery via thick blood smears, the cohort was divided into two groups, one consisting of women who had given birth once and the other consisting of women who had ≥ 2 deliveries. Surprisingly, the comparison showed on significant difference between the two groups (Pearson Chi square test). However, when primiparous women and secundiparous women were grouped together as pauciparous women and compared to those who had ≥ 3 deliveries, it was interesting to observe that the former group had a significantly higher infections rate. Consequently, all further analyses made in this study took into consideration comparisons not only between primi- and multiparous women but also comparisons between pauci- and multigravids.

Infection rates of women with documented malaria during pregnancy

In this analysis, it was also noted whether the recruited women had previous malaria infections during the course of their pregnancies, as opposed to the previous comparison whereby they were blood smear positive at the time of delivery. Unlike the results of the first comparison, where no significant difference was found in infection rates as determined at the time of delivery between primi- and multiparous women, this time the former group was in fact found to be significantly more infected than the latter group, when analysed for the presence of malarial infections during pregnancy (P = 0.02). There was also a significantly higher rate of infection in pauciparous women during pregnancy than in multiparous women (P = 0.006).

Recognition of variant surface antigens by flow cytometry

Placental isolates from two Gabonese (Gb337 and Gb218) and two Senegalese (vip43 and vip42) patients were used in flow cytometric assays to assess the level of VSA_{PAM}-specific IgG in the plasma of the 151 women recruited in this study as well as plasma from five control nulligravid women and seven males. Antibody reactivity was recorded as the mean fluorescent intensity (MFI) of the ethidium bromide positive cells determined in the assay. The parity of the 151 women ranged from 1–14 and, due to the low number of women with high parities, those with seven or more parities were grouped with the sixth parity group for statistical analysis purposes. The levels of antibodies recognizing placental isolates were found to correlate with parity (Figure 1). A Kruskal-Wallis non-parametric one-way analysis of variance was further applied to the data and has shown that there are differences among the medians of all six groups (P = 0.003). It was observed also that the mean rank score values in the test in all the parity groups (from the third birth upwards) were significantly higher when compared to the value in the primigravid group, but this was not the case when compared to the secundigravid group. These patterns were consistent with all four parasites tested.

On the other hand, plasma samples from women who had not had children were not reactive. In addition, sex-specific recognition of the placental isolates was also observed in the plasma from the 151 women, since male control samples also showed no recognition. The plasma samples correlated in their capacities to recognize surface antigens expressed on erythrocytes infected with different field isolates. The comparison was made based on MFI values after subtracting the mean + 2 standard deviations of the MFI values calculated from the nulli and male plasma samples. This value was calculated for each parasite isolate for each flow cytometric assay and all values were highly reproducible (MFI range between 30 and 60) in three independent experiment for each isolate. For all

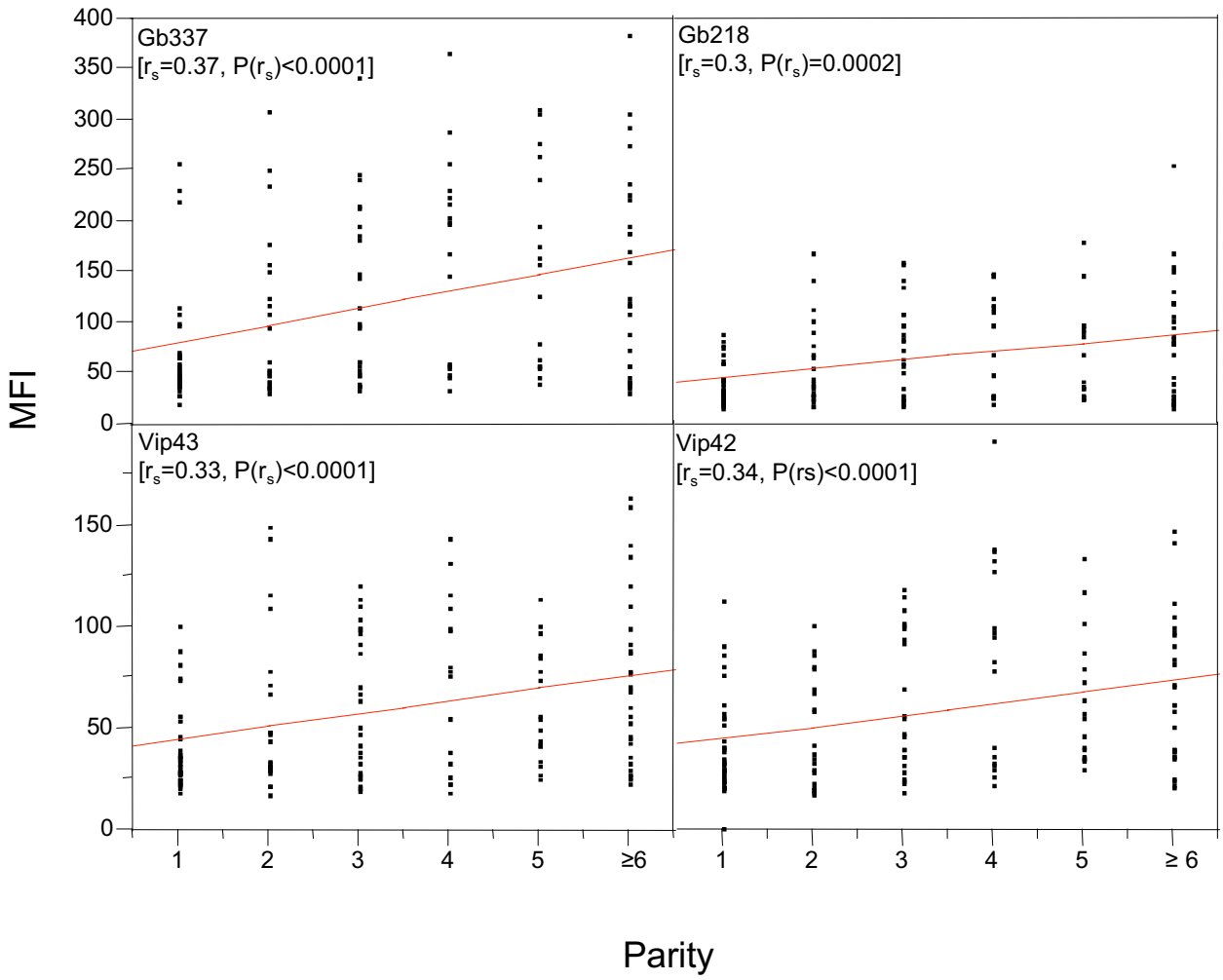

Figure 1
Parity dependence of recognition of variant surface antigens on four placental parasite isolates by VSA_{PAM}-specific IgG in plasma obtained from 151 women at delivery from an area of hyperendemic malaria transmission (Lambaréné, Gabon). Parasite isolate and the corresponding statistical significance of the correlation between parity and mean fluorescence intensity are shown. The fit line using least squares regression is shown for each relationship.

the pairs of parasite isolates, the levels of VSA_{PAM}-specific plasma IgG correlated, even though for certain pairs of comparison (such as that between parasite isolates Gb337 and vip42 or Gb337 and vip43) the correlation was less tight than for others (Fig. 2) (see discussion). Nevertheless, the level of correlation of plasma reactivity between different placental isolates is sufficiently significant to suggest a common nature of surface antigens expressed on infected erythrocytes causing PAM.

The effect of *P. falciparum* positive placentas on the levels of recognition was also investigated. Plasma taken from women of the primiparous group whose placentas were infected had higher levels of antibodies than plasma from women from the same group whose placentas were not infected, as shown for one parasite Gb337 (Fig. 3, panel A). The same pattern was observed for plasma taken from the pauciparous women (panel C). On the other hand, this was not found to be the case for the multiparous

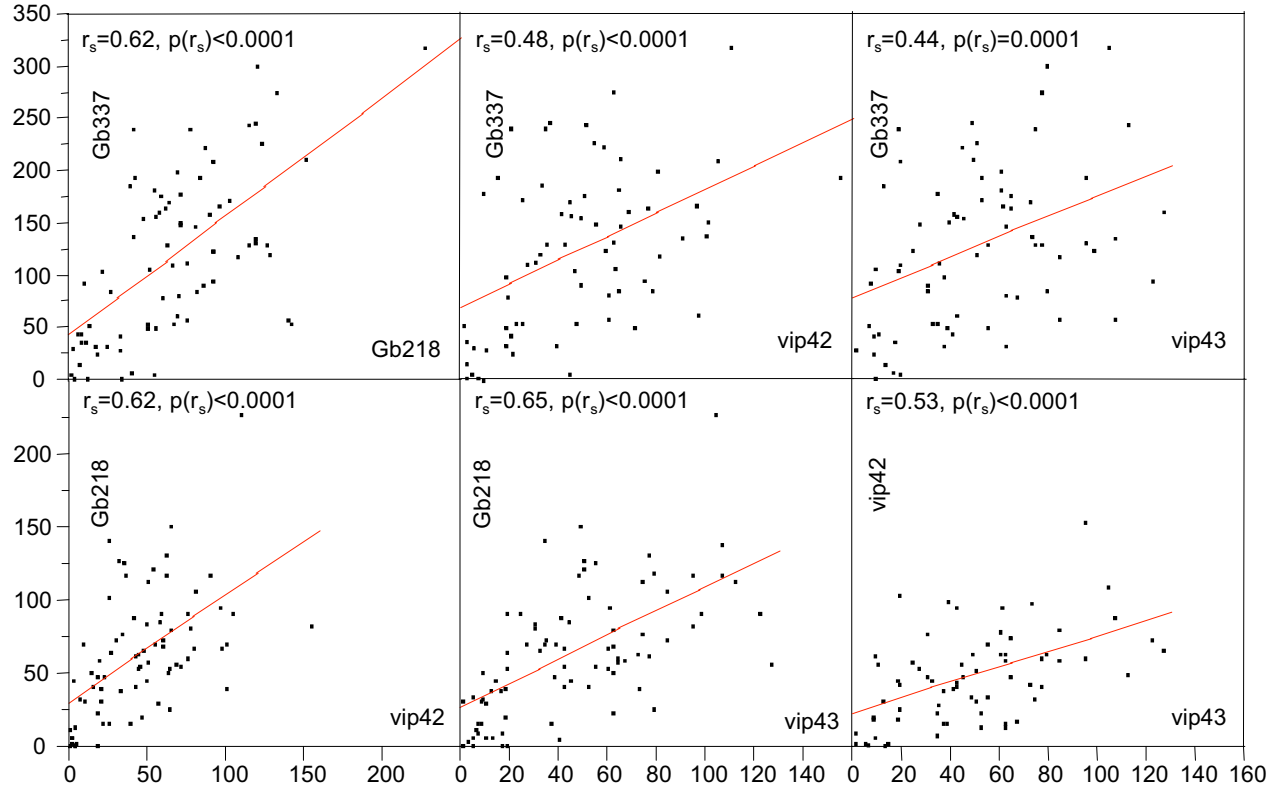

Figure 2
Relationship of anti-VSA levels between different placental isolates. Anti-VSA levels were measured as mean fluorescence intensity (MFI). Spearman rank correlation coefficients together with their strengths are given for each parasite pair. The fit line using least squares regression is shown for each comparison.

group (panels B and D). Here, women who have been pregnant more than twice generally have high antibody levels. For three out of the four parasites that were analysed in this study, similar patterns were observed. P values for the comparisons are given in Table 2.

The level of anti-VSA antibodies was compared in pauciparous women between those who had infected placenta at delivery and women with positive thick blood smear during pregnancy but with negative placenta at delivery. In the latter category, it was observed that their anti-VSA antibodies generally appeared to be lower for some of the tested isolates. P values of the statistical comparisons were 0.03 for Gb337, 0.05 for vip43, 0.08 for Gb218 and 0.35 for vip42. The same comparison made for primiparous women showed no difference.

Inhibition of binding assays

Based on the observation that some primiparous women have high levels of VSA_{PAM}-specific IgG as measured in flow cytometry, an effort was made to determine whether the antibodies found in this population of primiparous women, who also carried malaria positive placentas could provide some protection against malarial infections, for example by limiting parasite accumulation in the placenta. The capacity of these antibodies to inhibit the binding of erythrocytes infected with placenta parasites to CSA was therefore evaluated, CSA being the main receptor molecule on the syncytiotrophoblast cells lining the placenta implicated in PAM. Eight plasma samples of placenta positive women which exhibited high levels of recognition in flow cytometry were selected for analysis. A comparison of their MFI levels with their anti-adhesion capacities showed that six out of the eight plasma samples analysed also had high levels of anti-adhesion antibodies and were able to inhibit binding to CSA by 86 – 96 %,

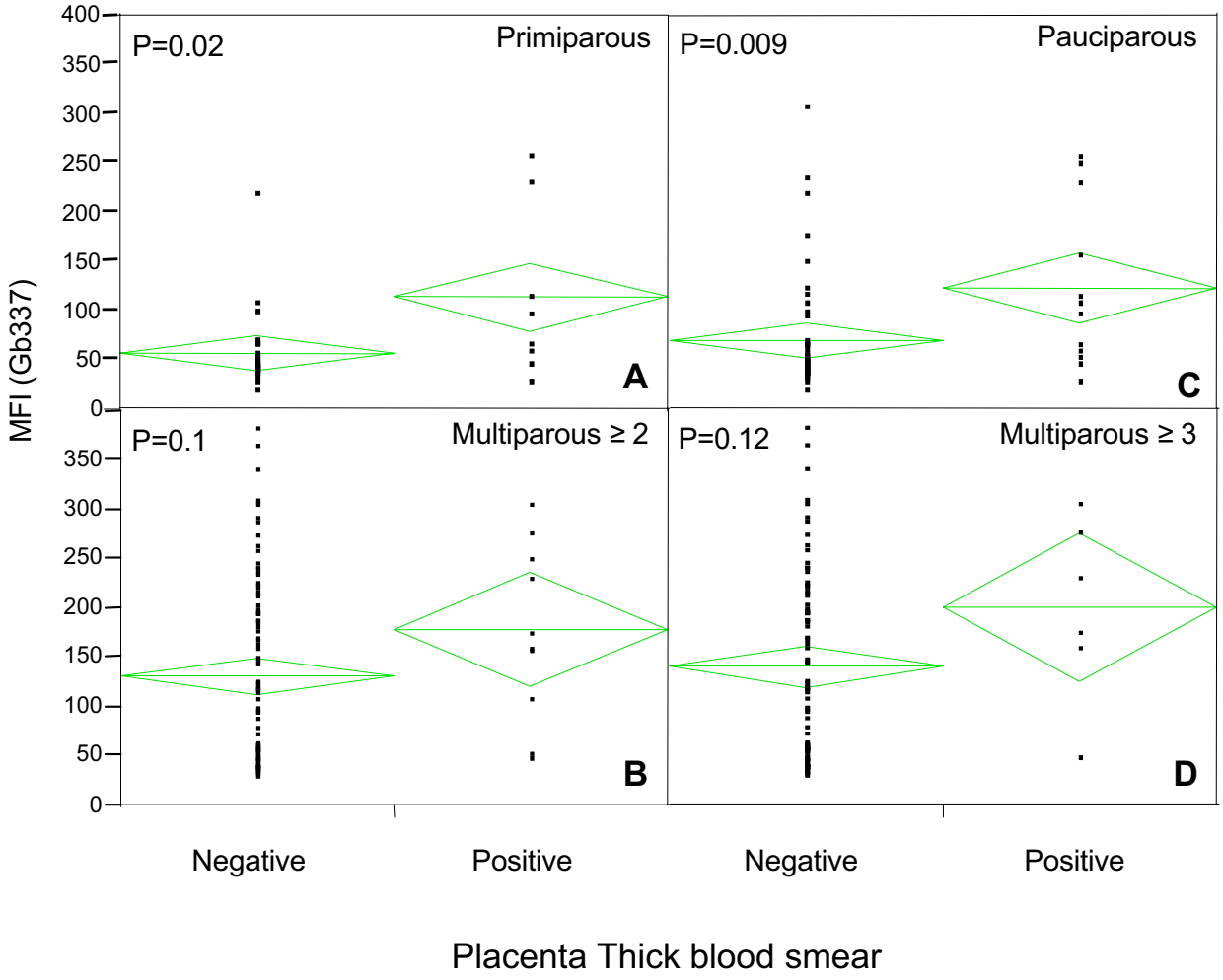

Figure 3
Effect of placental infections as determined by thick blood smears on the level of anti-VSA antibodies. The MFIs of plasma were obtained and compared between women who presented with positive and negative placentas in (A) primiparous women and (B) multiparous women (≥ 2). The same comparisons was made between positive and negative placentas in (C) pauciparous women and (D) multiparous women (≥ 3). The data showed here were performed on the parasite Gb337. P values for each comparison are indicated in the graphs and the grand means of all MFI values for each group are shown as dashed lines. The center line in each diamond shows the group mean, and the vertical spans of the diamond show the 95% confidence interval.

based on results of experiments carried out with placental parasite vip43 (Figure 4). Thus, the presence of malarial infection was observed to induce high levels of VSA_{PAM}-specific IgG in primiparous women. The correlation between levels of anti-PAM antibodies measured by flow cytometry and the anti-adhesion capacities of these antibodies implies a link between functional and antigenic domains.

Discussion

The observation that the rates of placental infections at delivery are similar between the primi-and secundigravid women in this area (Table 1) demonstrates that women in their second pregnancies are almost as susceptible to malarial infections as those who are pregnant for the first time. This result is in keeping with previously published work [25,26]. Another notable finding is that the

Table 2: Comparison of MFI levels of plasma from women with non-infected and infected placentas at delivery within each group using Wilcoxon rank test

	Mean rank score of non-infected/infected placentas (P-value)			
Placental parasite	Primiparous women	Multiparous women ≥ 2	Pauciparous women	Multiparous women ≥ 3
Gb337	18/29 (0.02)	54/71 (0.1)	30/45 (0.009)	43/59 (0.12)
Vip43	19/28 (0.04)	54/67 (0.2)	30/44 (0.01)	43/59 (0.12)
Gb218	19/29 (0.04)	53/72 (0.07)	29/42 (0.04)	43/62 (0.07)
Vip42	18/26 (0.08)	52/68 (0.1)	28/39 (0.07)	42/60 (0.08)

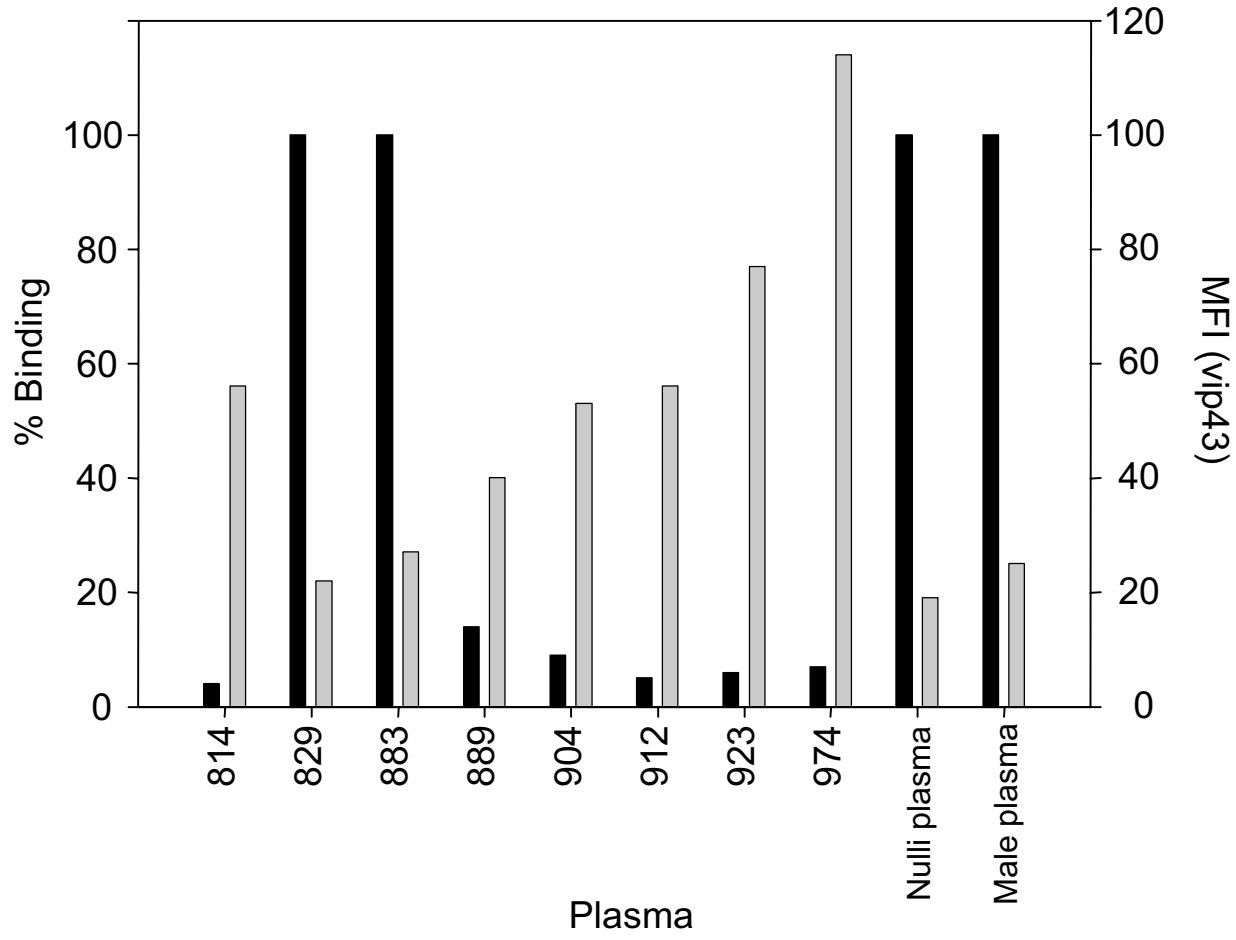

Figure 4
Comparison of antibody levels in surface recognition and CSA biding. CSA adhesion activities (■) and the corresponding anti-VSA levels (□) measured as mean fluorescent intensities of plasma obtained from primiparous women presented with infected placentas are shown. High CSA binding also stands for low anti-CSA adhesion activity. Plasma samples from a semi-immune nulligravid woman and a male served as controls.

percentage of primigravid women who are infected at delivery is not significantly higher than multigravid women, which could be due to differences in malaria transmission intensities in different study areas. The observed lack of significance is probably not solely due to the small sample size, since significance was achieved when primi- and secundigravid women were grouped together and compared to multigravids. In the study area, only 22 % of the primiparous women were infected at delivery, whereas in other areas, higher infection rates at first pregnancies have been reported, for example 38 % in Kisumu, Kenya [27] and 34–40 % in Shire Valley, Malawi [28].

However, when another parameter was investigated, namely the presence of malarial infections during pregnancy, there was a significantly higher percentage of primigravids who were infected compared to multigravids. Such an observation may be attributed to the time factor during data collection. While data collected at delivery is restricted to one point in time, that collected during pregnancy is accumulated over several months. Thus, those primigravid women who did not carry malarial infections at delivery, but who could have been infected during their pregnancies, would not have been included in this group. On the other hand, the chance of detecting an infection given a longer time span is simply higher.

The data of this work were generated using placental parasites collected from two different study areas and plasma samples collected from women delivering in Lambaréné. The parity-dependent pattern of recognition by plasma samples collected from pregnant women using placental isolates was not as obvious as that observed for laboratory parasite strains selected for CSA binding [14]. The reason may be that placental parasites appear to be less well recognized than CSA-selected parasites. Host factor may also play a role in recognition pattern differences thus it may be that immunity develops more slowly in Gabon due to low exposure. The results described here show that women in their second pregnancies still presented with low levels of anti-VSA antibodies which, in terms of their measured MFI levels were not significantly different from that measured in the primiparous group (Fig. 1). However, the levels of antibodies recognizing the surface of IEs were observed to increase significantly only from the third parity upwards, in comparison to the primigravid women. Since MFI obtained in the secundigravid group was not significantly higher than the primigravid group, it reinforces the observation that secundiparous women are probably as susceptible to malarial infection as the primigravid group. In this case, the grouping of secundiparous women with multiparous women is likely to skew the data.

Good correlation was observed between different placenta parasite isolates and their recognition by plasma samples from women of different parities (Fig. 2), indicating that the plasma antibodies are targeting the same family of antigens [29] responsible for placental malaria on infected erythrocytes. The less firm correlation between the parasite isolates Gb337 and vip42 or vip43 compared with other parasite pairs could be because the parasites were assayed against Gabonese sera, and Gb337 parasite is a Gabonese parasite while vip42 and vip43 are of Senegalese origin. However, difference in geographic origin alone may not sufficiently explain the variations in the correlation coefficients between Gb337/vip42 and Gb337/vip43 pairs and the rest of the comparisons, particularly since Gb218/vip42 and Gb218/vip43 yield a different picture. It is likely that Gb337 in being the most recognized parasite amongst the four parasites studied has shaped the recognition pattern and the variations observed are thus an isolate-specific phenomenon.

When data were segregated into whether placentas were infected or not at delivery and analysed as a function of parity (Fig. 3 and Table 2), it was obvious that sera from primiparous women with malaria-positive placenta had significantly higher mean MFI than those with negative placentas. Anti-VSA antibodies in the susceptible women (pauciparous), who were infected during pregnancy and negative at delivery, could be shorter-lived antibodies than in multiparous women. This could be explained by the possibility that multiparous women by being pregnant more often risk continuous exposure to infection and hence repeated boosting that could lead to an accumulation of memory against VSAs. This would in turn trigger a quick immune response following infection, resulting in an overall higher level of these antibodies (not apparent in primiparous women). Multiparous women therefore exhibited higher levels of anti-VSA antibodies regardless of whether the placenta infected or not. Beeson et al [17] reported very similar findings in which VSA_{PAM} specific IgGs were more prevalent in infected than uninfected primigravidae and their levels did not differ in infected and uninfected multigravidae. In an earlier study, Ricke et al [14] showed that a considerable number of primiparous women presented high levels of anti-VSA antibodies, but this was not correlated with placental infections, as samples were collected from the participants at their third-trimester. Other studies have also shown that all women, regardless of parity, produced VSA_{PAM}-specific IgG, determined as anti-CSA adhesion antibodies [15,30], contrary to the findings of Fried et al [13]. The latter showed that plasma from primigravid women, unlike that of multigravid women had little anti-adhesion antibodies against CSA-binding parasites. Such discordant findings may be explained by the use of different plasma dilutions or the setting of different cut-off values. In our

hands, plasma of primiparous malaria-infected women containing high anti-VSA antibodies were also capable of inhibiting the binding of placental parasites to CSA (Fig. 4). Beeson et al [17] have postulated that CSA-adhesion inhibitory antibodies and anti-VSA antibodies may be acquired independently during infection and have different roles in immunity. Results from our study based on isolate vip43 would suggest that the surface of erythrocytes infected with it simultaneously express overlapping functional and antigenic domains. The identification of functional antibodies which appear to inhibit the binding of parasites to CSA in the placenta is crucial to developing a successful vaccination strategy against PAM.

Conclusion

The results indicate that pauciparous women from the study area are more infected at delivery than multiparous women. Recognition of placental parasites shows a similar parity-dependency pattern, which is highly suggestive of the common nature of surface antigens expressed on IEs causing PAM. Primiparous women infected at delivery appear to carry antibodies capable of recognizing the surface of IEs and blocking their binding to CSA. The observation that pauciparous women with positive thick blood smear during pregnancy but with negative placenta at delivery carried lower levels of anti-VSA can be explained by the short life span of the induced antibodies. The finding of a correlation between serum reactivities of different placental field isolates would indicate that the parasite ligands on the surface of IEs involved in CSA binding and causing PAM are most likely to represent functionally conserved molecules of a distinct subfamily.

Authors' contributions

AK collected parasites from placentas, performed the flow cytometric assays, established the *in vitro* binding assays, performed the statistic analyses and drafted the manuscript. CR was responsible for plasma sample collection in Gabon and participated in the maintenance of the cultures and the flow cytometry analysis in the laboratory. TS and LH guided us in culturing of field isolates and in flow cytometry. PGK is in charge of the Research Unit of the Albert Schweitzer Hospital. NF provided us with parasite samples from Senegal, and PD supervised the field work in Senegal. MQK was responsible for the design and implementation of the study, as well as the management of the project throughout the study period.

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